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T cell recognition of Mls-like superantigens: analysis of TCR requirements, superantigenic ligands, and signal transduction

*Richard J. Hodes and Ryo Abe**

Mls-like endogenous superantigens have been reported to be unique, differing from conventional antigens in the T cell receptor (TCR) requirements for their recognition, in the signal transduction events which they elicit in T cells, and in the identity of the stimulatory ligand. This review describes recent findings indicating that TCR Vα as well as Vβ products play a role in Mls recognition. Data are also summarized which indicate that T cell recognition of Mls leads to increased intracellular [Ca^{2+}] and phosphatidyl inosotol hydrolysis. Finally, genetic linkage between mouse mammary tumor virus (MMTV) proviruses and endogenous superantigens is analyzed, and the possible role of non-MMTV products is examined.

Key words: T cell / T cell receptor / superantigen / MMTV

MLS (MINOR LYMPHOCYTE STIMULATING) antigens were first characterized by Hilliard Festenstein and his colleagues as antigens expressed by certain mouse strains which were capable of stimulating vigorous primary proliferative responses by T cells from major histocompatibility complex (MHC)-identical strains which did not express these determinants.[1] This unique ability to stimulate large proliferative T cell responses was the source of considerable interest for those interested in antigen recognition by T cells. Initially, Mls antigens Mls^a, Mls^c and Mls^d were regarded as the allelic products of a single genetic locus, for which the non-stimulatory allele was Mls^b. However, subsequent findings from our own laboratory as well as others revealed several surprising aspects of the Mls antigens. First, it was demonstrated by conventional genetic analysis that the stimulatory Mls determinants were not expressed on allelic products of a single locus. Rather, Mls^a and Mls^c determinants were the products of distinct and non-allelic genes, and the Mls^d phenotype represented the co-expression of the independently encoded Mls^a and Mls^c products.[2-7] Thus, there was no evidence for polymorphic allelism at any Mls locus, but rather the expression or non-expression of a T cell stimulatory product of each locus. Further analysis revealed another unique characteristic of Mls determinants, first demonstrated for Mls^c, namely a 'genetic redundancy' such that two or more unlinked genes existed either one of which was sufficient to encode expression of an indistinguishable Mls determinant.[8,9]

These unique characteristics of the Mls system, the absence of allelic polymorphism and the presence of genetic redundancy, were explained by the recent discovery by several laboratories of the relationship between Mls-like 'superantigens' and endogenous mouse mammary tumor virus (MMTV) proviruses.[10-12] Different proviruses, with distinct sequences in regions including the open reading frames of their LTRs, have different strain distributions.[10-13] Each provirus is integrated at one genomic site and is either present (giving an Mls stimulatory phenotype) or absent (the non-stimulatory phenotype), without structurally allelic forms of its product, consistent with the behavior of Mls determinants. In addition, distinct proviruses integrated in unlinked loci can share sufficient sequence homology to encode highly cross-reactive or indistinguishable Mls-like determinants,[13] accounting for the 'genetic redundancy' observed in the encoding of Mls.

The other recent development which has provided critical insights into the functional behavior of Mls antigens is the characterization of antigen recognition via the antigen-specific T cell receptor (TCR). Antigen recognition by most T cells is mediated by an antigen-specific receptor which is an αβ heterodimer, each chain of which is the product of multiple variable gene segments (V, D, and J for the β chain, and V and J for the α chain) as well as non-germline

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encoded junctional residues.[14] T cell recognition of conventional protein antigens involves recognition of peptides presented in association with self MHC molecules, and is dependent upon the precise combinatorial specificity of the TCR, requiring the appropriate co-expression of multiple variable α and β chain segments.[15] In contrast, a class of 'superantigens' has been described, the recognition of which appears to depend primarily upon expression of an appropriate Vβ product, with little or no influence of other TCR components. Thus, all or nearly all T cells expressing an appropriate TCR Vβ product are responsive to a superantigen. In addition to exogenous superantigens, which are mycoplasma, bacterial, or viral products, the Mls-like products expressed in the mouse represent a unique class of endogenous superantigens.[9,16-20] Each of the endogenous superantigens which has been characterized to date appears to be encoded by a distinct MMTV provirus, each of which has a unique distribution among mouse strains.[10-13] As noted above, many of the endogenous superantigens are capable of stimulating primary proliferative responses by T cells from mice that do not express the corresponding superantigen. In addition, these endogenous superantigens mediate the clonal deletion during T cell development of cells which express a Vβ product capable of reacting with that 'self' superantigen.[9-17,19-21]

This review summarizes our recent analysis of the ligands which serve as Mls-like superantigens, of TCR expression by Mls^a-reactive T cells, and of the nature of signal transduction events which are associated with T cell recognition of Mls^a.

Association of MMTV proviruses with deletion of T cells expressing Vβ5, 11, and 12

A group of Vβ families, which includes Vβ 5, 11, 12, and 16, was initially characterized as 'I-E' related in that most strains which express an EαEβ class II MHC product delete T cells expressing the products of these Vβ genes.[22,23] More recently, it was shown that the self ligands which mediate deletion of Vβ5, 11, and 12-expressing cells are determined by multiple non-MHC-linked genes[19,20,24] and it was subsequently reported that the ligand(s) for Vβ5 and Vβ11 map to MMTV proviruses Mtv-8, Mtv-9, and Mtv-11.[11,12] At least some of these Vβ-deleting MMTV proviral products also serve as superantigens in the Mls-like stimulation of primary T cell proliferative responses.[19,20] The relationship between specific TCR Vβ deletions and expression of particular MMTV proviruses was analyzed in more detail for this newly characterized set of superantigens. In order to relate Vβ specificity to MMTV LTR sequences, a series of backcross animals was analyzed in which individual Mtv-8, Mtv-9, and Mtv-11 proviruses detected by Southern blotting segregated independently, and in which MMTV expression could be correlated with deletion of Vβ5, 11, and 12. The linkage of specific MMTVs to these deletions was found to be complex. Expression of Mtv-9 was sufficient to delete essentially all mature T cells expressing Vβs 5, 11 and 12; Mtv-8 caused complete deletion of Vβ12, but only partial deletion of Vβ11, and little or no deletion of Vβ5; and Mtv-11 caused complete deletion of Vβs 11 and 12, but no deletion of Vβ5 (Figure 1). If these differences in Mtv-8,9, and 11 are related to sequence differences in the C-terminus of the LTR open reading frame, as suggested by previous studies,[13] the limited sequence differences among these three MMTVs either produce different determinants for Vβ-specific recognition or lead to affinity differences in recognition of the same determinant by different TCR Vβ products.[25]

T cell receptor Vβ repertoire in the absence of MMTV proviruses

The effect of endogenous proviruses on the developing TCR repertoire is considerable, potentially resulting in the deletion of large portions of the potential repertoire. This has stimulated considerable speculation upon the potential advantages or disadvantages of such effects for the host.[25,26] To date, however, the existence of endogenous superantigens has been established only in the mouse. In addition, each of the superantigens which has been identified in the mouse has been associated with a MMTV provirus. An important question, which remains to be clarified, is therefore whether endogenous superantigens are limited to the MMTV proviruses expressed in mice, or whether they represent a more generalized biological phenomenon. We have addressed this issue by two approaches, the first of which involved the study of feral mice that do not express detectable MMTV proviruses, and the second of which examined the ability of another species, the rat, to contribute to the ligands for Vβ-specific negative selection in mice.

	Vβ5	Vβ11	Vβ12
Mtv-8	No deletion	Partial deletion	Complete deletion
Mtv-9	Complete deletion	Complete deletion	Complete deletion
Mtv-11	No deletion	Complete deletion	Complete deletion

Figure 1. Relationship between TCR Vβ expression and MMTV provirus. Segregation analyses were carried out in multiple strain combinations in which the presence or absence of multiple MMTV genomic proviruses segregated independently. Individual mice were then analyzed for genomic MMTV and for TCR Vβ expression. The presence of proviral Mtv-8, Mtv-9, and Mtv-11 in individual mice was correlated with deletion of Vβ5, 11, and 12 as summarized.

The Czech II strain was derived from feral mice and has previously been shown to lack expression of any integrated MMTV provirus when assayed by Southern analysis.[27] Mice of this strain were therefore analyzed for their TCR Vβ expression to determine whether they might express non-MMTV ligands capable of mediating Vβ-specific deletion. Vβ expression in CD4+ and CD8+ Czech T cells

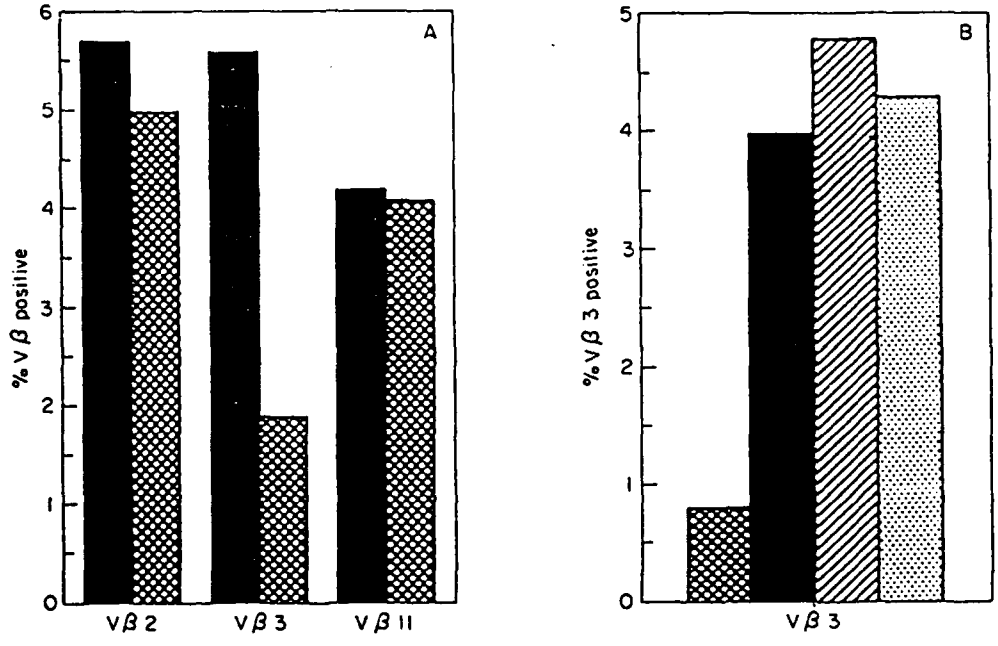

Figure 2. T cell receptor Vβ expression by CD4+ T cells in MMTV provirus-negative Czech II mice, in their F_1 offspring, and in B10.A mice foster-nursed by Czech II females. Results are expressed as the percent of CD4+ spleen cells that express the indicated TCR Vβ product. ■, B10.A; ▩, Czech II; ▨, (B10.A × Czech) F1; ▧, B10.A-F-Czech.

was compared with expression in the strain C57BL/10 (B10), which undergoes no known Vβ-specific deletions due to its failure to express an MMTV product with a permissive class II MHC product, or with the strain B10.A which does exhibit a number of MMTV-dependent Vβ-specific deletions. Czech T cells expressed a wide profile of Vβ products, in general at levels similar to those observed in B10 T cells. However, Vβ3$^+$ T cells were expressed at significantly lower levels on both CD4$^+$ (Figure 2A) and CD8$^-$ (data not shown) Czech T cells than on the corresponding B10 or B10.A populations. Two approaches were taken to determine whether this reduced level of Vβ3 expression was the result of negative selection during T cell development. First, the expression of Vβ3 during intrathymic T cell development was compared in thymocytes from Czech, from non-Vβ3-deleting B10.A, or from known Mls^{c+} Mtv-1$^+$ Mtv-6$^+$ C3H/HeJ.[10] As previously reported,[9] superantigen-mediated Vβ3 deletion in C3H/HeJ thymocytes occurred at the transition from immature cells expressing a low density of TCR to mature cells expressing a high TCR density, so that Vβ3-bright cells were selectively deleted whereas there was a much smaller effect on Vβ3-dull thymocytes. In contrast, Czech thymocytes exhibited similarly reduced frequencies in Vβ3-bright and Vβ3-dull populations, suggesting that the reduced levels of Vβ3 expression did not result from clonal deletion during intrathymic maturation (data not shown).

In order to further assess the expression of a Vβ3-deleting ligand in the Czech genome, F_1 mice were generated by breeding Czech males with females of the B10.A strain, a strain which is known to have an MHC type permissive for Vβ3 deletion, but which does not express a Vβ3-specific MMTV ligand. These F_1 mice failed to delete Vβ3, indicating that the Czech genome does not encode a ligand capable of mediating Vβ3 deletion in these animals (Figure 2B). In contrast, when F_1s were constructed between Czech males and females from the Mls^{c+}, Mtv-1$^+$ Mtv-6$^+$ strain C3H/HeJ, these (C3H/HeJ × Czech) F_1s deleted Vβ3 at the nearly complete level of deletion characteristic of the C3H/HeJ parental strain (data not shown). To test the possibility that a milk-borne agent (such as an infectious MMTV) carried by Czech mice, and not a genomically transmitted product, could mediate Vβ3 deletion, B10.A mice were foster-nursed on Czech females and the resulting mice analyzed. No Vβ3 deletion was observed in these foster-nursed animals, thus failing to detect a milk-borne Vβ3-deleting agent (Figure 2B). Collectively, these data indicate that MMTV-free Czech mice fail to express a detectable Vβ-deleting ligand and are consistent with the conclusion that Vβ-specific T cell clonal deletion is a unique property of MMTV-dependent ligands.

Effect of rat bone marrow-derived cells on the mouse T cell receptor Vβ repertoire

The existence of Vβ-specific clonal deletion has to date been demonstrated only in the mouse. Specific attempts to identify a similar phenomenon in the rat have failed to detect Vβ-specific deletion.[28] In man, individual differences in Vβ expression have been identified, but have not been shown to result from ligand-specific events in T cell selection. To test the possibility that endogenous products of mammalian species other than mouse might mediate Vβ-specific deletion, an approach was designed using mixed radiation bone marrow chimeras. Lethally irradiated C57BL/10 (B10) mice were reconstituted with either B10 mouse bone marrow alone or with a mixture of B10 and rat bone marrow.[29] Mice reconstituted with B10 mouse bone marrow alone did not exhibit any Vβ-specific deletions in their reconstituted T cell repertoires (Figure 3). In contrast the B10 bone marrow-derived T cells of chimeras which had been reconstituted with a mixture of B10 and WF strain rat bone marrow demonstrated specific deletion of Vβ5$^+$ and Vβ11$^+$ T cells; chimeras which had been reconstituted with F344 strain rat marrow did not show such deletion (Figure 3). Thus, rat bone marrow derived cells were able to contribute in a strain-specific manner to the ligand for Vβ-specific deletion in mouse T cells. The identity of the rat gene product involved in this specific deletion of mouse T cells was not established in these experiments. All of the Vβ-specific deleting ligands described to date have both MHC class II components and non-MHC-linked superantigen components. Thus, rat bone marrow-derived cells may have contributed an I-E-like class II molecule which functioned to present mouse MMTV proviral products capable of mediating Vβ5 and Vβ11 deletion; alternatively, WF strain rat cells may express distinct and presumably non-MMTV ligands capable of mediating Vβ5 and Vβ11 deletion. The presence or absence of Vβ-specific deletions in the TCR repertoire expressed by rat cells in these chimeras is now under investigation.

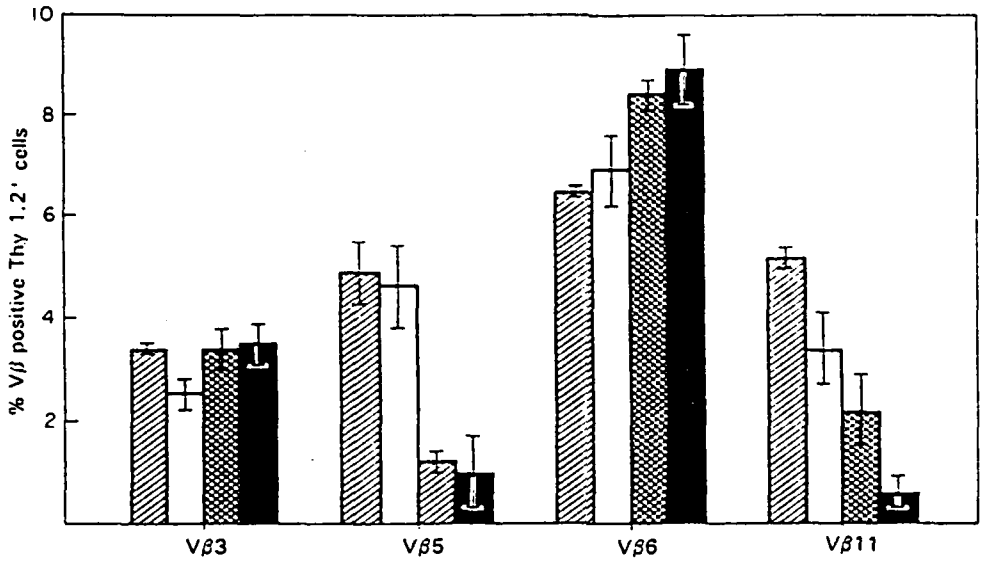

Figure 3. Contribution of rat bone marrow-derived cells to TCR Vβ-specific T cell deletion in radiation bone marrow chimeric mice. Radiation bone marrow chimeras were prepared by injecting lethally irradiated recipients with bone marrow of the indicated rat and/or mouse strain. Mouse bone marrow-derived Thyl.2+ T cells were analyzed for expression of the indicated TCR Vβ product. Figure reproduced from the *Journal of Experimental Medicine*, 1992, 175: 147-157 by copyright permission of the Rockefeller University Press. ▨, B10; □, B10 + F344→B10; ▩, B10 + WF→B10; ■, BR→BR.

Influence of TCR Vα expression on Mls^a reactivity

As noted above, Mls and Mls-like superantigens have been identified primarily by their ability to mediate Vβ-specific clonal deletion of developing T cells or Vβ-specific clonal activation of mature T cells. The analysis of natural and transfected Vβ variants has further analyzed the role of Vβ sequence in recognition of Mls superantigens, and has suggested that superantigen recognition is dependent upon regions of the TCR that are distant from those involved in recognition of conventional antigens.[30] However, two lines of evidence have suggested that TCR specificity for endogenous Mls superantigen is not determined by the expressed Vβ product alone. Yui *et al* reported that some but not all T cell clones expressing a transgenic Vβ8.1 β chain were Mls^a (Mtv-7) reactive,[31] and Pullen *et al* found that the ability of a transfected Vβ8.2 β chain to mediate Mls^a reactivity in hybridomas was influenced by the α chain co-expressed.[32] In order to formally assess the role of α chain expression in Mls^a reactivity, the pairing of specific Vα products with Vβ6 was analyzed in populations of Mls^a responsive T cells. T cells from Mls^a-negative mice were stimulated *in vitro* with Mls^a-positive cells, or with control allogeneic but Mls^a-negative cells, or with anti-TCR antibodies. These T cell populations were then analyzed by two-color flow cytometry for their simultaneous expression of Vβ and Vα products. When T cells from Mls^a-negative strains were activated *in vitro* for 6 days with Mls^a-expressing allogeneic stimulator cells, a marked selective expansion in Vβ6$^+$ cells was observed, reflecting the dominance of Vβ6-expressing T cells in the response to Mls^a.[33] When Vα expression was analyzed on these expanded Vβ6$^+$ T cells, it was found that the distribution of specific Vα products was substantially different from that on Vβ6$^+$ T cells which had not been selected for Mls^a responsiveness.[34] Mls^a-responsive Vβ6$^+$ T cells showed significantly decreased frequencies of

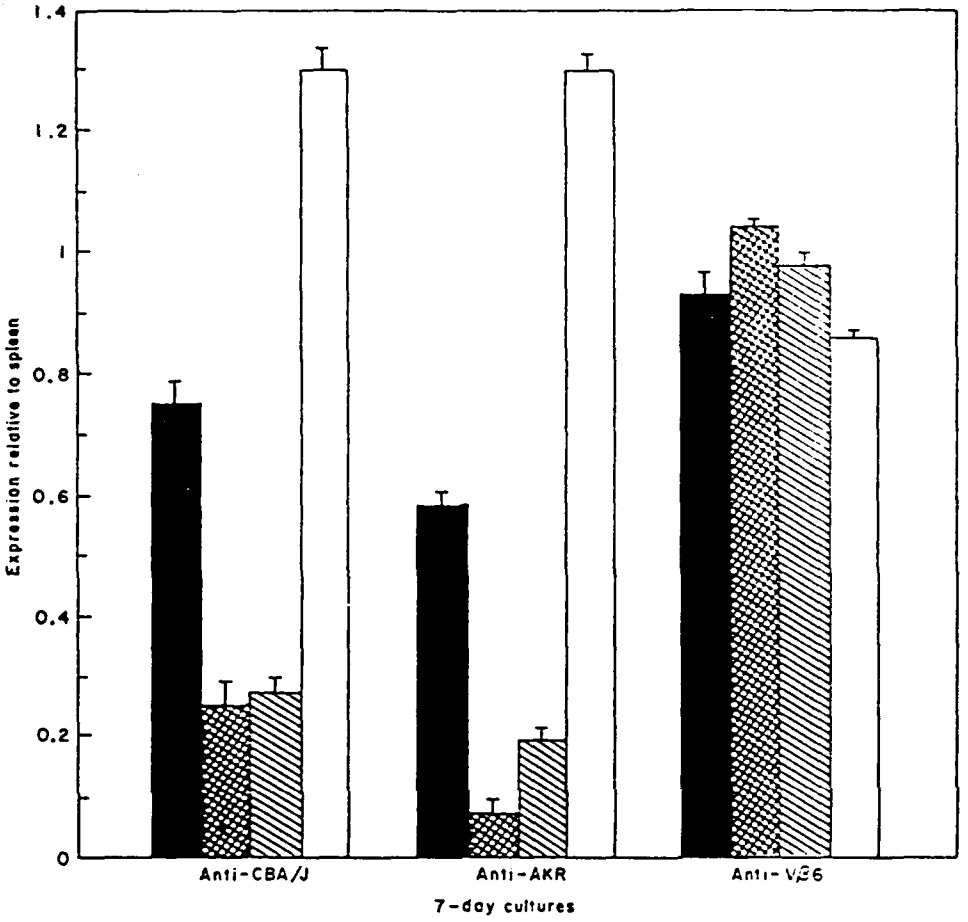

Figure 4. Influence of TCR Vα expression on reactivity of Vβ6⁺ T cells to Mls^a. T-enriched spleen cells were cultured with the indicated stimulus for 7 days prior to analysis for Vα expression in Vβ6⁺ T cells and were compared to freshly isolated spleen cells. TCR expression in the cultured cells was expressed relative to that seen in uncultured Vβ6⁺ spleen cells:

$$\% \text{ of expression in spleen} = \frac{\text{Vα/Vβ6 in 7-day cultures}}{\text{Vα/Vβ6 in spleen cells}} \times 100.$$

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expression of Vα2, 3, and 8 and an increase in expression of Vα11 when compared with unstimulated Vβ6⁺ T cells (Figure 4). This pattern was consistent in the responses of different strains to Mls^a stimulators and was specific for Mls^a responses in that no similar skewing of Vα usage was observed after stimulation of T cells with non-Mls alloantigens or with anti-Vβ6 antibody. Thus, T cell responsiveness to Mls^a is not a function of Vβ usage alone, but is substantially influenced by expression of specific Vα products as well.

Since Vα expression influences the *in vitro* response of mature T cells to Mls^a, attempts were made to determine whether Vα expression also exerts an influence on Vβ-specific negative selection by Mls superantigens. Attempts to study Vα expression on any residual Vβ6⁺ cells which might remain undeleted in Mls^a-expressing mice were unsuccessful

due to the very small number of such cells. The completeness of this deletion itself suggests that essentially all $V\beta6^+$ cells, regardless of $V\alpha$ expression, are deleted in the presence of endogenous Mls^a product. Partial $V\beta$ deletion has been described in the case of $V\beta11$ deletion in A/J mice, apparently mediated by the Mtv-8 proviral ligand. $V\alpha$ expression was therefore analyzed in the $V\beta11^+$ T cells found in A/J and in the non-$V\beta11$-deleting MHC congenic strain A.BY. No difference in $V\alpha$ expression was observed with the two $V\alpha$-specific antibodies reactive with this strain. There was therefore no indication that $V\alpha$ plays a role in the incomplete negative selection of $V\beta11$ in A/J mice.

These findings suggest something of a paradox in that there is an apparent influence of $V\alpha$ expression on the *in vitro* response of mature T cells to an Mls superantigen, but there may not be a similar influence on negative selection mediated by the same ligand. It should be noted, however, that the systems in which these two effects are measured are quite different. The expansion of $V\beta6^+$ cells during *in vitro* T cell responses to Mls^a reflects the selective survival and/or expansion of certain Mls^a-reactive T cells. These cells may, for example, represent those with an optimal TCR affinity for the Mls^a ligand. The selective $V\alpha$ expression in these cells is therefore characteristic of the selected population of cells which are most capable of proliferation or survival in response to Mls^a. In contrast, it is possible that negative selection *in vivo* does not reflect the same requirements; a T cell may be deleted after intrathymic interaction with an Mls^a product at some threshold level of affinity, despite the fact that the TCR expressed by this cell might not mediate a competitively strong proliferative response to that ligand *in vitro*. The existence of differential requirements for negative selection versus peripheral T cell responsiveness mediated by the same TCR has in fact been reported in mice transgenic for a TCR specific for lymphocytic choriomeningitis virus plus class I D^b.[35]

Signal transduction events mediating T cell responses to Mls^a

The precise nature of T cell stimulation by endogenous Mls-like superantigens is unknown. Substantial evidence exists supporting a role for the $\alpha\beta$ TCR and for CD4 expressed by the T cell and for class II MHC product on the stimulatory APC. However, as discussed above, the molecular characteristics of endogenous superantigens are unknown, and it is unclear whether signals transmitted via the TCR in response to these superantigens are identical to those transmitted in response to conventional peptide antigens presented by self MHC class II molecules. O'Rourke *et al* have in fact concluded, on the basis of studies of heterogeneous T cells and T cell clones, that stimulation of T cells by Mls^a induces proliferation by a pathway that does not involve detectable phosphatidyl inosotol (PI) hydrolysis, whereas stimulation of the same T cells by MHC-encoded alloantigens does induce PI hydrolysis, leading to the conclusion that Mls^a stimulation is mediated by a distinct signal transduction pathway.[36] Similar conclusions were reported by Gaugler *et al*[37] and Patarca *et al*.[38] To characterize the signaling events which are involved in T cell recognition of Mls^a, the response of Mls^a-specific T cell clones to splenic stimulator cells derived from Mls^a-positive or negative stimulators were first studied. Cloned T cells were loaded with the $[Ca^{2+}]$-sensitive dye indo-1 and stimulator cells were labeled with the dye $DiI_{22}(3)$. T cells and stimulator cells were then mixed, and the cell mixture was analyzed by flow cytometry. It was observed that Mls^a-specific T cells formed a greater frequency of cell-cell conjugates with Mls^a-positive than with Mls^a-negative cells, indicating that Mls^a-specific conjugates were being formed.[39] In addition, those cloned T cells which were conjugated with Mls^a-positive cells demonstrated a rapid increase in intracellular $[Ca^{2+}]$ after warming to 37 °C (Table 1). The same comparison also demonstrated a specific increase in PI hydrolysis after interaction of cloned T cells with Mls^a stimulators.

Table 1. Intracellular $[Ca^{2+}]$ response of cloned T cells to Mls^a stimulation

Stimulator cells	H-2	Mls^a (Mtv-7)	$[Ca^{2+}]_i$ increase*
AKR.J	k	+	+
CBA/J	k	+	+
D1.LP	b	+	+
DBA/2	d	+	+
CBA/CaH	k	–	–
C3H/HeJ	k	–	–
B10.BR	k	–	–
C57BL/6	b	–	–
B10.Q	q	–	–

*Positive responses were increases in $[Ca^{2+}]_i$ of greater than 200 nM compared to pre-stimulation values. Negative responses were increases less than 15 nM.

These results indicated that T cell recognition of Mls^a results in both increased intracellular [Ca^{2+}] and PI hydrolysis, suggesting that the PLC-dependent pathway of activation can be induced by Mls^a recognition in cloned Mls^a-specific T cells. In order to determine whether similar signaling occurs in the recognition of Mls^a by normal heterogeneous T cells, peripheral T cells were prepared from Mls^a-positive AKR/J mice, from Mls^a-negative CBA/CaH mice, and from transgenic CBA/CaH mice expressing an Mls^a-specific TCR Vβ8.1 transgene product on greater than 95% of peripheral T cells. These T cells were indo-1 loaded and mixed with labeled Mls^a-positive or negative spleen cells. It was observed that T cells from Mls^a-negative mice formed cell-cell conjugates with Mls^a-positive stimulator cells, and that a high proportion of T cells in these conjugates underwent a significant increase in intracellular [Ca^{2+}]. T cells from normal Mls^a-negative mice formed reactive conjugates in a proportion consistent with that expected for Mls^a-reactive cells in heterogeneous populations; T cells from Vβ8.1 transgenic mice responded in a correspondingly high proportion.[39] Thus, for cloned and normal heterogeneous T cell populations, recognition of the Mls^a superantigen appears capable of inducing increased intracellular [Ca^{2+}] as well as increased PI hydrolysis, potentially reflecting TCR-mediated PLC activation.

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